

Stavrianopoulos et al.

Serial No.: Not Yet Known

(Divisional of S.N. 10/096,075, filed March 12, 2002)

Filed: Herewith

Page 3 [Preliminary Amendment (Accompanying Divisional Application  
Under 37 C.F.R. §1.53(b)) --- January 23, 2004]

**PLEASE AMEND THIS APPLICATION AS FOLLOWS:**

**In The Title:**

Change the title of the invention to:

-- PROCESS FOR DETECTING THE PRESENCE OR QUANTITY OF  
ENZYMATIC ACTIVITY IN A SAMPLE --

**In The Claims:**

Please cancel claim 1.

Please add new claims 287-305 as follows:

Claim 1 (Canceled Herein)

Claims 2-286 (Previously Canceled)

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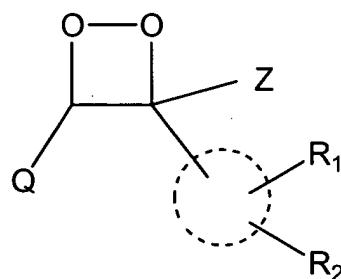
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287. (NEW) A process for detecting the presence or quantity of enzymatic activity of interest in a sample, said process comprising the steps of:

(a) providing:

- (i) said sample suspected of containing enzymatic activity;
- (ii) a chemiluminescent reagent having the structure:



wherein Q comprises a cycloalkyl or polycycloalkyl group attached covalently to the 4-membered ring portion of said dioxetane above directly or indirectly through a linkage group; wherein Z comprises hydrogen, alkyl, aryl, aralkyl, alkaryl, heteroalkyl, heteroaryl, cycloalkyl or cycloheteroalkyl; and wherein R<sub>1</sub> and R<sub>2</sub> comprise chemical moieties attached to different sites of a cyclic ring attached to said dioxetane, and wherein R<sub>1</sub> is enzymatically converted into R<sub>1</sub>\* which comprises a chemical reactive group G<sub>1</sub>, and wherein R<sub>2</sub> is attached to said cyclic ring through an oxygen atom and comprises a chemical reactive group G<sub>2</sub> which reacts with said G<sub>1</sub> to convert said dioxetane to an unstable light-emitting dioxetane form.

(ii) reagents and buffers for carrying out chemiluminescent reactions;

(b) forming a mixture of:

- (1) (i), (ii) and (iii); or

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- (2) (ii) and (iii) and contacting said mixture of (ii) and (iii) with (i);  
(c) enzymatically converting the chemiluminescent reagent of (a)(ii)  
into an unstable light-emitting dioxetane form; and  
(d) measuring the quantity of light generated by said enzymatic  
conversion in step (c).

288. (NEW) The process of claim 287, wherein in said providing step (a) Q in said chemiluminescent reagent (ii) comprises an adamantyl group.

289. (NEW) The process of claim 287, wherein in said providing step (a) R<sub>2</sub> in said chemiluminescent reagent (ii) comprises a substituted or unsubstituted aliphatic group or an unsubstituted aromatic group.

290. (NEW) The process of claim 289, wherein said substituted aliphatic group comprises halogen, nitrates, sulfonates or nitrites.

291. (NEW) The process of claim 287, wherein said enzymatic converting step (c) is carried out by a substrate comprising amides, esters, phosphates, carboxylic acids, fatty acids, glucose, xylose, fucose, or amino acids.

292. (NEW) The process of claim 287, wherein said enzymatic activity of interest comprises an amidase, an esterase, an acetylcholinesterase, an acid phosphatase, an alkaline phosphatase, a decarboxylase, a lipase, a glucosidase, a xylosidase, a fucosidase, a trypsin or a chymotrypsin.

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293. (NEW) The process of claim 287, further comprising the step of forming an intermediate five- or six-membered ring comprising a linkage between said G<sub>1</sub> and G<sub>2</sub> in said chemiluminescent reagent (ii).

294. (NEW) The process of claim 287, wherein any of said steps (a) through (d) are carried out in liquid phase or mixed phase.

295. (NEW) The process of claim 287, wherein said enzymatic activity of interest is dependent upon the presence or quantity of another compound.

296. (NEW) The process of claim 295, wherein said another compound comprises an RNA or DNA probe.

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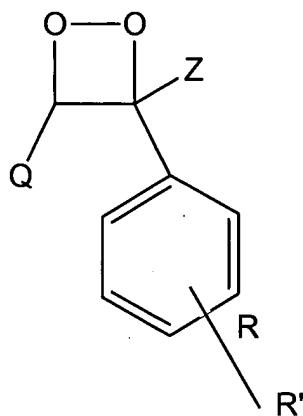
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297. (NEW) A process for detecting the presence or quantity of enzymatic activity of interest in a sample, said process comprising the steps of:

(a) providing:

- (i) said sample suspected of containing enzymatic activity;
- (ii) a chemiluminescent reagent having the structure:



wherein Q comprises a cycloalkyl or polycycloalkyl group attached covalently to the 4-membered ring portion of said dioxetane above directly or indirectly through a linkage group; wherein Z comprises hydrogen, alkyl, aryl, aralkyl, alkaryl, heteroalkyl, heteroaryl, cycloalkyl or cycloheteroalkyl; and wherein R comprises a chemical linker having a reactive site attached to the aromatic ring in said structure; and wherein R' comprises a substrate for a non-cleaving enzymatic process, wherein the product of said enzymatic process leads to further chemical rearrangements that generate an unstable light emitting dioxetane form.

(ii) reagents and buffers for carrying out chemiluminescent reactions;

(b) forming a mixture of:

- (1) (i), (ii) and (iii); or

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- (2) (ii) and (iii) and contacting said mixture of (ii) and (iii) with (i);
- (c) enzymatically converting the chemiluminescent reagent of (a)(ii) into an unstable light-emitting dioxetane form; and
- (d) measuring the quantity of light generated by said enzymatic conversion in step (c).

298. (NEW) The process of claim 297, wherein in said providing step (a) Q in said chemiluminescent reagent (ii) comprises an adamantyl group.

299. (NEW) The process of claim 297, wherein in said providing step (a) R in the chemiluminescent reagent (ii) comprises a substituted or unsubstituted aliphatic group or an unsubstituted aromatic group.

300. (NEW) The process of claim 299, wherein said substituted aliphatic group comprises halogen, nitrate, sulfonate or nitrite.

301. (NEW) The process of claim 297, wherein said providing step (a) R in the chemiluminescent reagent (ii) comprises a reactive site comprising an oxygen, a nitrogen or a sulfur atom.

302. (NEW) The process of claim 297, wherein said step of enzymatically converting (c) is carried out by an enzyme comprising an oxidase or reductase.

303. (NEW) The process of claim 297, wherein any of said steps (a) through (d) are carried out in liquid phase or mixed phase.

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304. (NEW) The process of claim 297, wherein said enzymatic activity of interest  
is dependent upon the presence or quantity of another compound.

305. (NEW) The process of claim 304, wherein said another compound comprises  
an RNA or DNA probe.

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